Osteoarthritis and Cartilage (2001) **9,** 152–160
© 2001 OsteoArthritis Research Society International
doi:10.1053/joca.2000.0370, available online at http://www.idealibrary.com on IDEAL®
1063–4584/01/020152+09 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

More knee joint osteoarthritis (OA) in mice after inactivation of one allele of type II procollagen gene but less OA after lifelong voluntary wheel running exercise

T. Lapveteläinen*, M. Hyttinen*, J. Lindblom†, T. K. Långsjö*, R. Sironen*, S.-W. Li‡, M. Arita‡, D. J. Prockop‡, K. Puustjärvi§ and H. J. Helminen*

**Department of Anatomy, and †Department of Applied Zoology and Veterinary Medicine, University of Kuopio, 70211 Kuopio, Finland; ‡Center for Gene Therapy, School of Medicine, MCP Hahnemann University, Philadelphia, Pennsylvania, U.S.A.; §Department of Physical Medicine and Rehabilitation, Kuopio University Hospital, 70211 Kuopio, Finland*

Summary

Objective: To investigate the incidence and severity of osteoarthritis (OA) and the effects of voluntary wheel running in normal mice and mice carrying either a targeted inactivation of one allele, heterozygous 'knockout', of Col2a1 gene or both alleles, homozygous 'knockout', of Col11a2 gene.

Methods: Mice lived until 15 months of age in individual cages. Running activity was recorded around the clock. OA changes were evaluated from serial knee joint sections by light microscopy.

Results: Heterozygous inactivation of Col2a1 gene coding for type II procollagen made the cartilage more susceptible to OA. At 15 months of age, OA prevalence was 60–90% in knockouts and 20–45% in normal controls (P<0.01–0.001). Unexpectedly, a reduction of OA due to wheel running was observed in both knockout strains (P<0.05–0.01). This effect was most evident in the femoral condyles. Incidence of OA in runners was approximately 50–85% of that in sedentary littermates. OA prevalence was higher in normal control and runner mice with high body weight. Running did not affect OA development in normal mice.

Conclusion: Heterozygous knockout of Col2a1 gene increased the OA prevalence in mice. Lifelong voluntary wheel running had a protective effect against OA in both knockout mice lines. The reason for this remains unknown. Reduction of OA may result from the reorganization and strengthening of the articular cartilage collagen network and/or adjacent muscles due to running, or lower body weight. Increased compliance of the articular cartilage and bones of the knockout mice may also contribute to the reduction of OA in exercised animals.

Key words: Inbred C57BL mice, Knockout mice, Articular cartilage, Cartilage diseases, Osteoarthritis, Aging, Running, Exercise.

Introduction

There is evidence for a genetic predisposition in a subset of patients with osteoarthritis (OA). Some mutations of collagen genes produce early-onset OA that may or may not be associated with features of a mild chondrodysplasia[1–9]. Osteoarthritis and other structural changes of articular cartilage have been investigated in mouse strains carrying transgenes which either disturb type II procollagen formation or cause a targeted inactivation of, e.g., type II collagen[10–14], the fibril associated type IX collagen[15–20], and the fibrillar type XI collagen genes[21].

In humans, increased body weight is associated with increased prevalence of knee joint OA and, at present, obesity is regarded as a definite risk factor for OA[22,23]. The intensity of physical activity has been shown to correlate with the incidence of OA. Recreational light sports appear not to increase OA, but strenuous physical work, requiring repetitive kneeling and squatting, or strenuous sports activities with the possibility of joint injuries and dynamic joint instability, seem to be risk factors[24–31]. On the other hand, running training of light intensity and relatively short length improves the biological properties of canine articular cartilage[32–34]. The relationship between OA and aging is well documented. However, it is not known to what extent the pathogenesis of OA shares common pathways with other age-associated dysfunctions, or whether OA is a time-dependent disorder distinct from normal aging with separate causative mechanisms working at the genetic, metabolic, behavioral, or environmental levels[35,36].

Spontaneously occurring murine OA and the effects of forced running exercise and aging on the development of OA in mice and other small laboratory animals have been discussed earlier[37]. Briefly, aging and forced running exercise increased the incidence of OA in normal mice. The effects of voluntary wheel running on murine articular cartilage and OA have not been assessed earlier. The purpose of the present study was to find out if the inactivation of either one allele of Col2a1 gene coding for proα1(II) chain or the homozygous 'knockout' of both alleles of Col11a2 gene coding for proα2(XI) chain were able to alter the strength of murine knee joint articular cartilage. We

Received 5 January 2000; accepted 16 June 2000.
Address correspondence to: Heikki J. Helminen, M.D., Ph.D., Department of Anatomy, University of Kuopio, 70211 Kuopio, Finland. Tel: +358-17-163000; Fax: +358-17-163032; E-mail: heikki.helminen@uku.fi

assumed that a reduced expression of Col2a1 gene would lead to an increase in OA incidence and severity. We also assumed that voluntary wheel running would accelerate and worsen these changes. Mice lived up to 15 months of age. The osteoarthritis scores were evaluated using light microscopy from knee joint sections[37] and the running activity of the mice was monitored throughout the experiment[38].

Method

Three genetically different inbred lines of mice were bred and held in the National Laboratory Animal Center (Kuopio, Finland). The normal C57BL/6JOlaHsd breeders were obtained from Harlan CPB (Rijswijk, The Netherlands) and the two lines of knockout breeders [C57BL/6-TGN(Col2a1 knockout Ht) and Col11a2 knockout Hm] originated from the Laboratory Animal Services of Thomas Jefferson University (Philadelphia, PA, U.S.A.). Knockout breeders were produced using homologous recombination technique[12]. Breeders heterozygous for Col2a1 allele, one allele inactivated[12], were mated with normal C57BL/6JOlaHsd mice to obtain either normal or heterozygous pups. The genotypes were verified using polymerase chain reaction (PCR) analysis as previously described[39]. Toe tips from 3-day-old and tail tips from 3-week-old (at weaning) mice were used as samples. The primers for Col2a1 analyses were 5′-GCT ATC AGG ACA TAG CGT TGG-3′, 5′-GGA GTC AGA GCA CTG GTC ATG-3′, 5′-CTG TTG CTT ATA GGA CTC AGG-3′, the inactivation of Col11a2 was confirmed with primers 5′-TTC ACC CTT GTG GCC CTT CA-3′, 5′-CTG AGG AGT CTT CAG ACT GG-3′ and 5′-AAT CCA TCT TGT TCA ATG GCC-3′. Missing toe tips served also as proofs of identity. Male mice were selected for the study. Eight experimental groups were formed from normal and heterozygous Col2a1 knockout mice: 9-month-old runners and controls as well as 15-month-old controls and runners. Breeders homozygous for Col11a2 allele, both alleles inactivated[21], were mated with each other to produce homozygous pups. Male mice were selected for the study. Two groups were formed from the homozygous Col11a2 knockout mice, 15-month-old controls and runners. Every experimental group consisted of 15–20 mice. The study protocol was approved by the Animal Care and Use Committee of the University of Kuopio.

After weaning, the pups lived together with the littermates for 1 week and were then, at the age of 1 month placed in individual cages 25×50×15 cm in size. Standard conditions prevailed[38]: temperature 21±2°C, humidity 50±20%, 12/12 h light rhythm, R36 Mouse Food (Lactamin AB, Stockholm, Sweden) and water *ad libitum* until sacrifice. The mice were weighed monthly. Runner mice had free access to running wheels within their cages (breadth 8 cm and diameter 23 cm). The running activity of the mice on the wheels was assessed using infrared sensors connected to a computer[38]. The running activity was measured for 23 h every day. The remaining hour from 10 a.m. to 11 a.m. was reserved for data storage and maintenance of the cages. Normally, the mice were idle and sleeping during that time. The measurements consisted of recording the running distance, running speed, and running time for any defined time period longer than 1 s. The calculated results were the average daily running distances and running speeds. Total lifetime running distances were also calculated.

The health of the mice remained excellent throughout the study. There was no mortality in the 9-month groups, and only one normal control mouse died at the age of 9 months from the 15-month group of animals of Col2a1 line. During the specimen preparation and sectioning of knee joints, five specimens out of 139 were discarded due to technical problems. There was no statistically significant difference between the treatment or age groups in mortality or number of lost specimens (chi-squared test). The number of mice which were evaluated for osteoarthritis/entered the study were as follows: 9-month-old normal controls 19/20 (95.0%), 9-month-old knockout (Col2a1) controls 20/20 (100%), 9-month-old normal runners 19/21 (90.5%), 9-month-old knockout (Col2a1) runners 18/19 (94.7%), 15-month-old normal controls 14/15 (93.3%), 15-month-old knockout (Col2a1) controls 15/15 (100%), 15-month-old normal runners 13/14 (92.9%), and 15-month-old knockout (Col2a1) runners 15/15 (100%). From 15-month-old Col11a2 knockout homozygotes, 15/15 (100%) of controls and 15/15 (100%) runners were investigated.

The mice were anesthetized by an intraperitoneal Avertin-injection (tribromomethanol and 2-methyl-2-butanolamyl alcohol, Sigma, St Louis, MO, U.S.A. and Aldrich Chemicals, Steinheim, FRG), X-rayed, and sacrificed by decapitation. The left knee joints were dissected free by removing the muscles, and by cutting off the femoral bone in the middle and the tibial and fibular bones in the distal end. The fixation and processing of the knee joints to paraffin blocks, cutting the sections in frontal direction, and the analysis of osteoarthritic changes were performed as described earlier in detail[37]. Using a conventional light microscope, the articular cartilage lesions were graded as: 0, intact cartilage; 1, superficial fibrillation; 2, deep defects extending to uncalcified cartilage; 3, defects extending to calcified cartilage; and 4, defects extending to the subchondral bone. These changes are illustrated in Fig. 1. The grading was performed in four cartilage compartments of the knee joint: medial and lateral condyles of femur and tibia. Three sections, approximately 200 μm apart, consecutively, were evaluated from every knee joint. The area assessed consisted of the cartilage residing between the frontal planes of insertions of the anterior and posterior cruciate ligaments, i.e. central areas of the tibial condyles and the corresponding areas in the femoral condyles (knee joint flexed in 90°). The structures were easy to identify from serial sections. The differences between the experimental groups were analysed using Mann–Whitney's U-test.

Results

During their lifetime, the normal runners of Col2a1 line (weight at 15 months 37.7±1.4 g; mean±S.E.M.) remained 2–10% leaner (P=0.011 at six months) compared to normal controls (38.7±1.4 g), and the knockout runners (weight at 15 months 37.4±1.6 g) 5–14% leaner (P=0.009–0.019 between 6 and 9 months) compared with knockout controls (39.5±1.3 g) (Fig. 2). The weight difference between the runners and controls reached the peak between 8 and 10 months and diminished after that. Normal controls remained 2–7% leaner than the knockout controls throughout the study. In Col11a2 line the knockout runners (weight at 15 months 28.0±0.6 g; mean±S.E.M.) remained 5–11% leaner (P=0.001 at 6 months) compared to controls (29.5±0.8 g). Col11a2 mice were smaller than Col2a1 mice

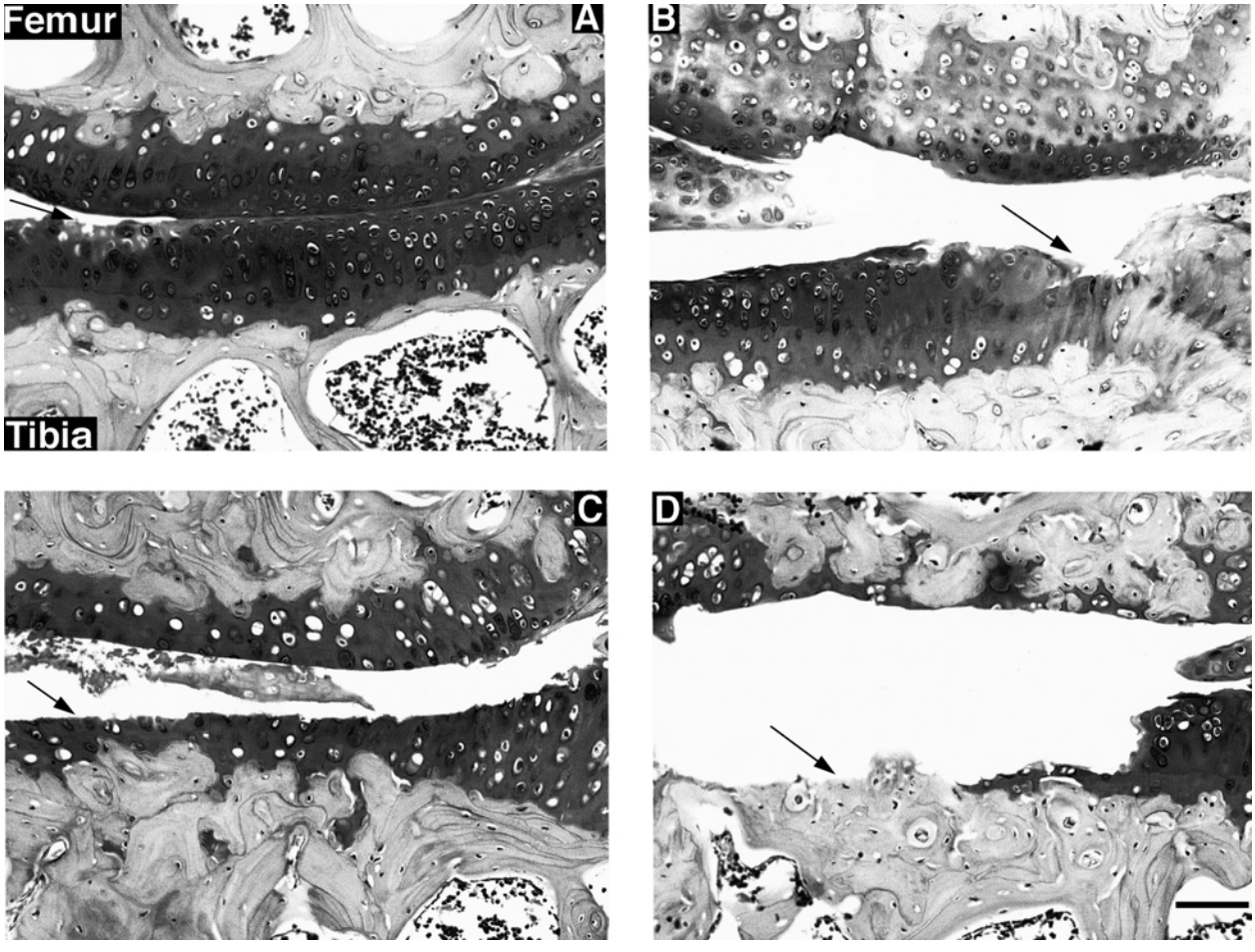

Fig. 1. Scoring of osteoarthritic (OA) changes from frontal knee joint sections of mice. In each picture the arrow points to the grade in question. A: grade 1—OA, superficial fibrillation, cell loss from the superficial zone and striation of the cartilage. B: grade 2—OA, deeper defects extending in the intermediate and deep zones of uncalcified cartilage. C: grade 3—OA, defects extending in the calcified cartilage. D: grade 4—OA, lesions extending in the subchondral bone. Safranin-O-staining. Bar=100 μm.

judged from weight (28.0-29.5 g vs 37.4-39.5 g, respectively). In this study, the consumption of food and water was assessed in the 9-month groups of Col2a1 mice. Consumption of food as well as water averaged between 30 and 40 g per week. The normal runners ate 8% more and drank water 13% more than the normal controls, whereas the knockout runners consumed 5% more food and water than the knockout controls.

In all groups, running was most active between the third and fourth months of age (Fig. 3). Normal mice of Col2a1 line ran up to 5 km and the knockouts up to 4 km a night. During the following months, the running distance of the normal mice remained 35–93% higher (P=0.013–0.041 from 6 to 15 months) than in knockouts. The running speed reached its peak during the third month, which roughly represents the time mice reach their musculoskeletal maturity. The running speed of normal mice was 7–16% higher (P=0.017 to 0.022 from 4 to 7 months and P=0.001 to 0.037 from 9 to 14 months) compared to the knockout mice. Despite their smaller size, the running activity of Col11a2 knockout mice was comparable and resided between the running activities of Col2a1 controls and knockouts (Fig. 3). Quite large variations existed in the activity of individual mice within the experimental groups, as denoted by the large standard deviations (S.D.) up to 40%. Total lifetime wheel running between mice varied between 580 and 2374 km (Table I). No correlation could be observed between the OA scores and total lifetime running activity or the changes in running activity during the last month, last 3 months, or last 6 months in any of the experimental groups. However, there was a statistically significant positive correlation between the variation of body weight and OA score in the medial tibial condyles (R=0.673, P=0.008) of the 15-month-old normal control mice and also in the medial tibial (R=0.588, P=0.034) and medial femoral condyles (R=0.691, P=0.009) of the 15-month-old normal runner mice. No other correlations were found.

At the age of 9 months, there was more OA in the knee joints of Col2a1 knockout mice, both in controls and in runners, compared with normal controls and runners (Fig. 4). The difference was most pronounced, with statistical significance (P=0.042–0.050), at the lateral tibial compartment. Running did not have any significant effect at this age. At the age of 15 months, the incidence of OA rose in every group of Col2a1 mice (Fig. 5). The increase was most pronounced in the femoral condyles of the knockout animals. A statistically significant difference (P<0.001) was observed between the normal controls and knockout controls, the latter having more OA. Importantly, there was not a similar difference between the normal runners or controls and the knockout runners. In the femoral condyles, there was a statistically significant reduction (P=0.002–0.048) in

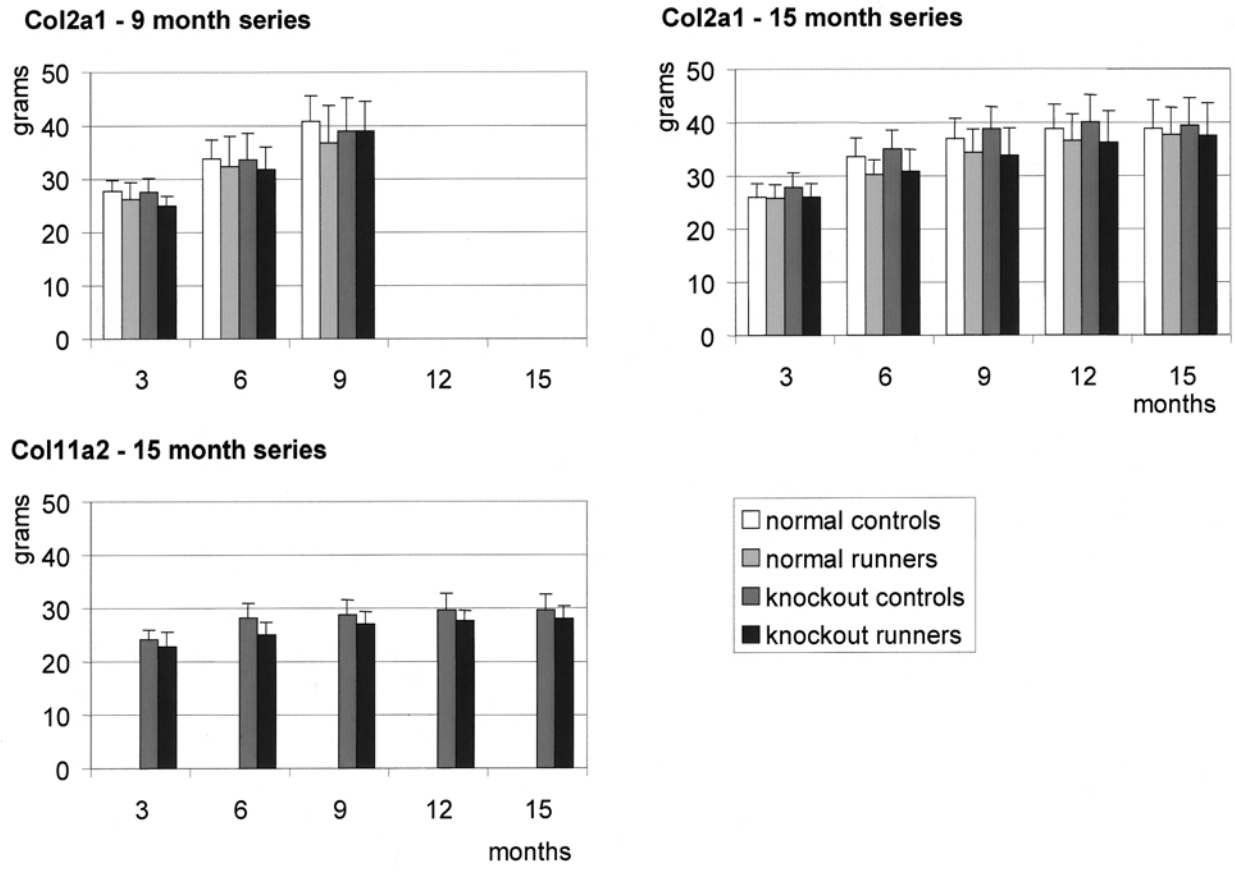

Fig. 2. Weight development of mice (mean±S.D.).

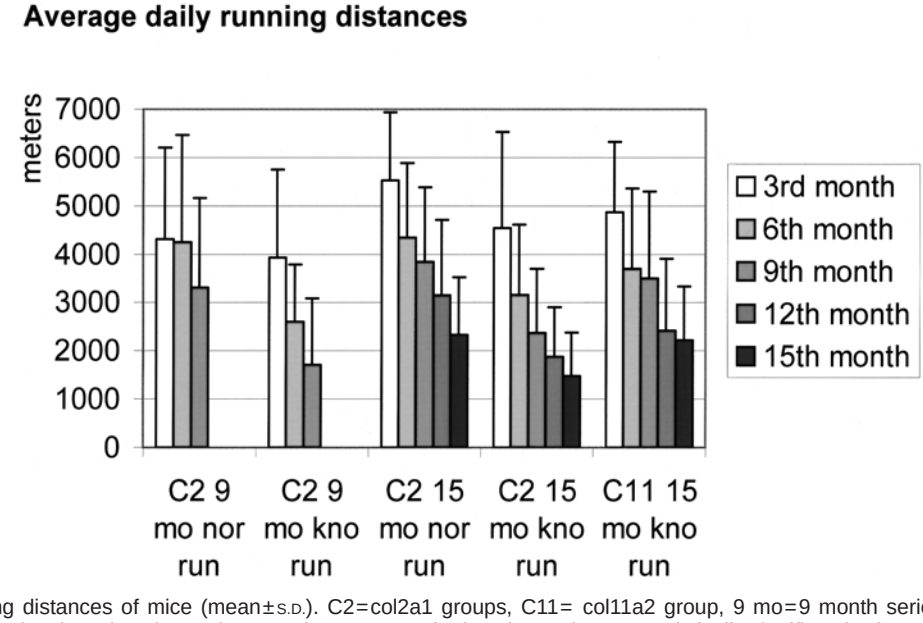

Fig. 3. Daily running distances of mice (mean±S.D.). C2=col2a1 groups, C11= col11a2 group, 9 mo=9 month series, 15 mo=15 month series, nor=normal mice, kno=knockout mice. In col2a1 groups, the knockout mice ran statistically significantly shorter daily distances from the sixth month on.

the rate of OA in favor of knockout runners compared with knockout controls (Fig. 5).

In Col11a2 knockout mice, the overall incidence of OA was the same or slightly lower than in the Col2a1 knockout mice (Fig. 5). Fifteen percent of these mice had severe osteoarthritic changes. Also in Col11a2 homozygous knockout mice, less OA was observed in the runners compared to sedentary knockout controls. This difference

Table I
Life-time running distances (mean±S.D.)

Group	Distances (km)	Minimum (km)	Maximum (km)
Col2a1 normal runners	1475±486	760	2374
Col2a1 knockout runners	1022±434	564	1860
Col11a2 knockout runners	1278±518	580	2245

was statistically significant in the lateral femoral condyles (P=0.041, Fig. 5).

Discussion

The inactivation of one of the two alleles of type II procollagen (Col2a1) gene disturbs only slightly the development and properties of murine cartilage tissue, even though the chondrocytes of the newborn mice synthesize only about half amount of the proα1(II) chains compared with the chondrocytes from wild-type animals[12]. In embryos and newborns, the development of cartilage and mineralization of bone primordia were at about the same stage in controls and knockouts judged from the Alcian Blue- and Alizarin Red-stained skeletons. The bone growth slightly lags behind in the knockouts, probably due to the minor alterations observed in the growth plate cartilage[12]. The differences in bone growth were less apparent as the mice grow older. Thus, in the articular cartilage of the heterozygous Col2a1 knockout mice, synthesis of normal proα1(II) chains and formation of normal type II collagen fibrils take place but to a lesser extent than in control cartilage[12]. In transgenic mice expressing a structurally altered proα1(II) chain together with normal chains, the alterations were more severe[13,14]. Mild or severe chondrodysplasias and early OA are among the syndromes observed in these mice. The mutated proα1(II) chains incorporate into the procollagen molecule destabilizing the collagen triple helix[4,5]. This results in a degradation of both the abnormal and normal type II procollagen molecules in a process called 'the procollagen suicide'[4,5]. Compared to the transgenic mice which produce the structurally altered proα1(II) chains with cellular changes, OA and chondrodysplasia[13,14], the heterozygous Col2a1 knockout mice appear to develop a milder phenotype with only minor alterations in the cartilage and bone. This is consistent with our observation that in the head of the humerus of these mice, the articular cartilage was softer and the collagen fibril network of cartilage showed reduced birefringence in the intermediate and deep zones (P<0.01–0.05) (Hyttinen *et al.*, unpublished observations).

Mice with a complete lack of proα2(XI) chain synthesis after inactivation of the Col11a2 gene (homozygous 'knockout') also show a minimal phenotype with few alterations[21]. The knockout mice are smaller than their littermates and

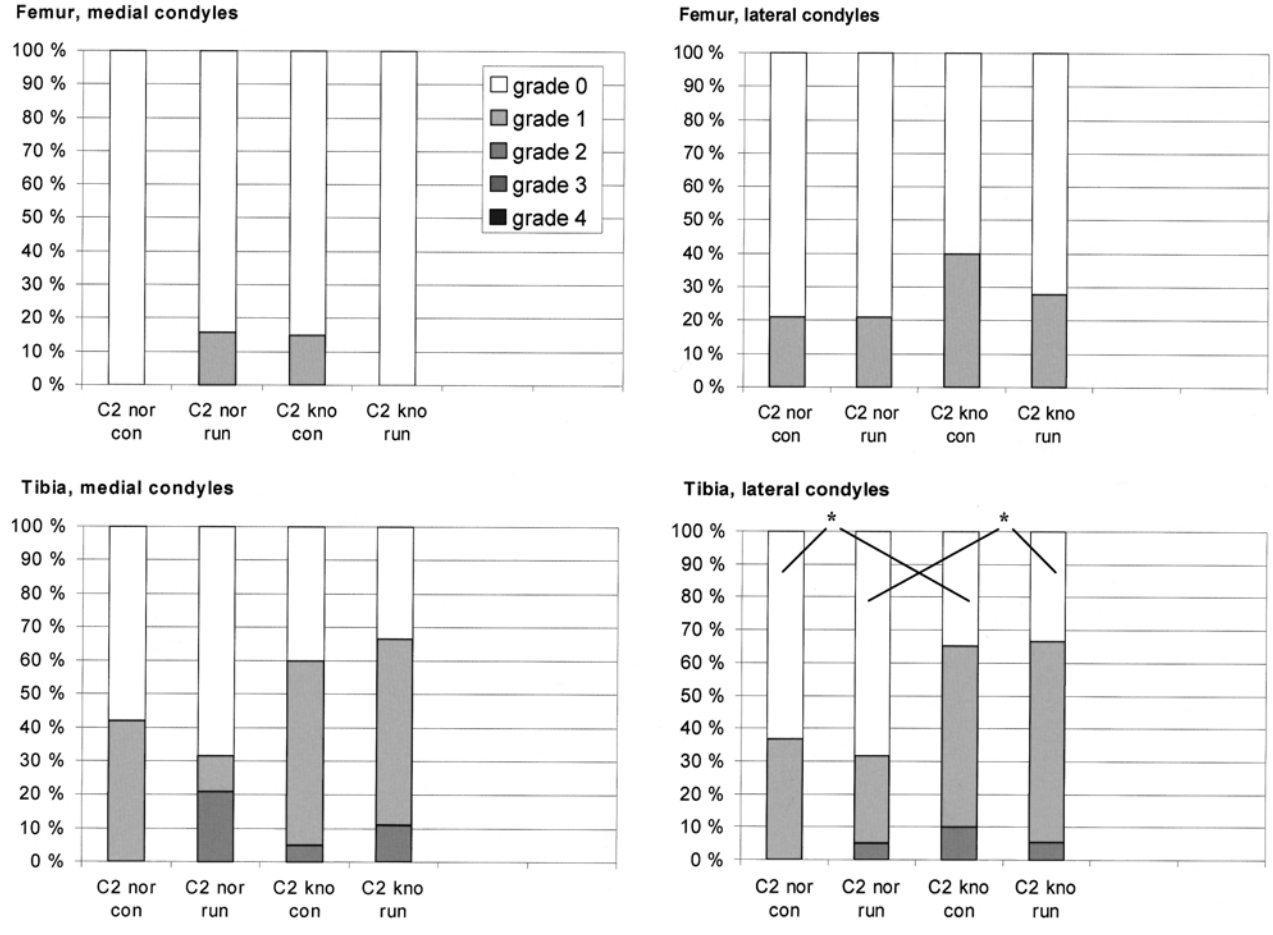

Fig. 4. The osteoarthritic changes in the knee joints of mice at nine months of age. C2=col2a1 groups, C11=col11a2 groups, con=controls, run=runners, nor=normal mice, kno=knockout mice. *=P<0.05, Mann–Whitney's U-test.

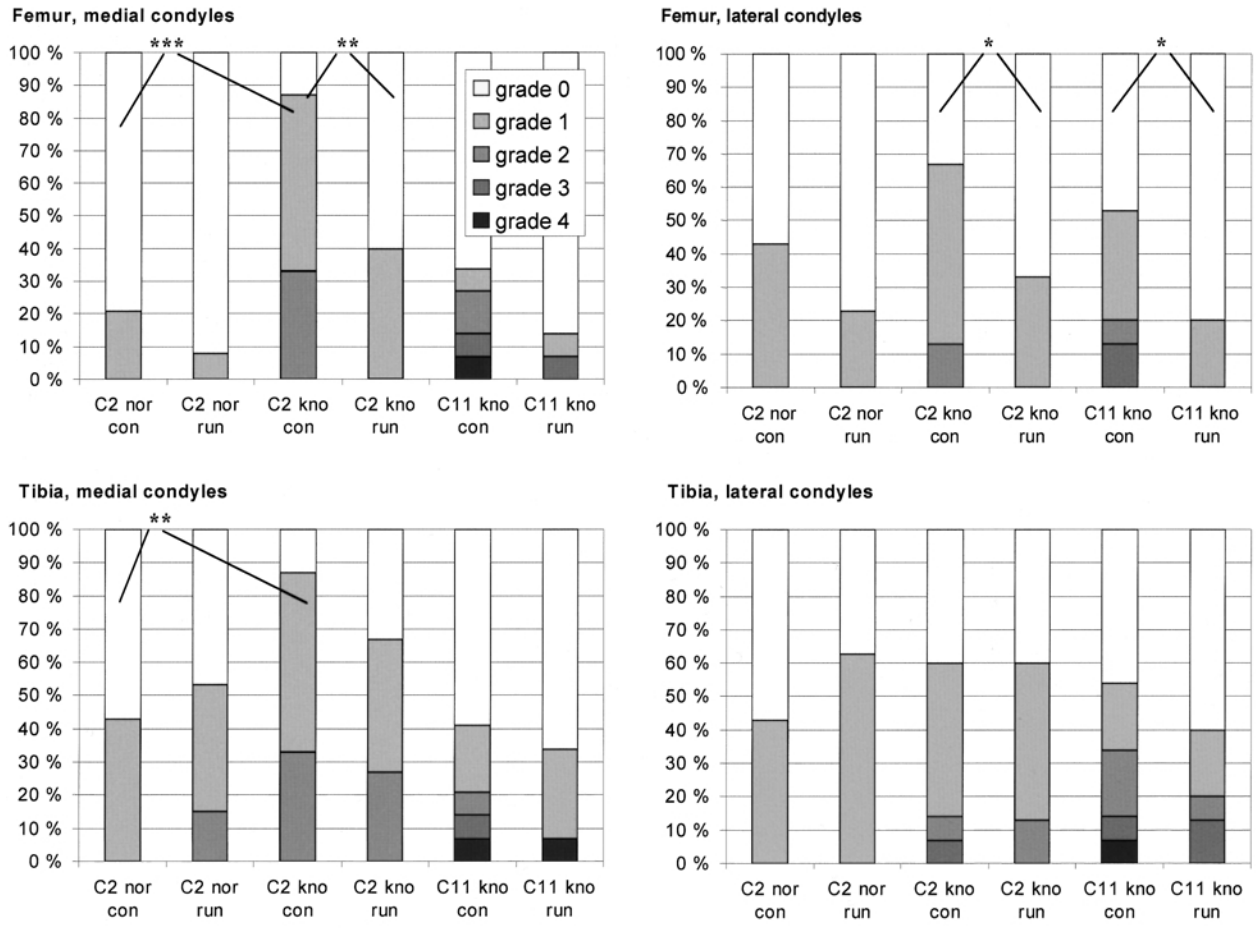

Fig. 5. The osteoarthritic changes in the knee joints of mice at 15 months of age. C2=col2a1 groups, C11=col11a2 groups, con=controls, run=runners, nor=normal mice, kno=knockout mice. *=P<0.05, **P<0.01, ***P<0.001, Mann–Whitney's U-test.

they have shorter and thicker bones[21]. By light microscopy, the growth plates at the end of long bones appear to be markedly disorganized. At the age of seven months, the changes of articular cartilage are few and there are no clear signs of OA[21]. Much stronger phenotypic changes take place after mutation of Col11a1 gene in cho/cho mice: abnormally thick collagen fibrils are found, as well as cleft palate, short spine and bones, and completely disorganized growth plates[3,40].

In humans, mutations of COL2A1 gene have resulted in chondrodysplasias characterized by short-limbed dwarfism and skeletal abnormalities, early-onset familial OA and milder forms of chondrodysplasias, spondyloepihyseal dysplasia, hereditary arthro-ophthalmopathy—Stickler syndrome, type 1 (STL1), and Wagner syndrome with ocular involvement only[1,3,9]. In STL1, the mutation in COL2A1 results in a premature stop codon in a COL2A1 allele, the probable outcome is a haploinsufficiency of the type II collagen reminiscent of the situation in the heterozygous knockout mice of this study. The function of type XI collagen in the articular cartilage matrix has not been conclusively established[8]. It may take part in the assembly of type II collagen fibrils to limit the fibril diameter, or form a core around which the fibrils are assembled. The third explanation is that type XI collagen fibrils bind to the surface of preformed type II collagen fibrils and prevent their aggregation when the cartilage undergoes physical compression. Nonocular form of Stickler syndrome, type 2 (STL2), has been associated with mutations of COL11A2 gene in humans[1,3,41]. In Stickler syndrome, a reduced synthesis of proα2(XI) chains has been observed[1,5]. The human syndromes are associated with reduced mRNA and specific protein synthesis.

Response of articular cartilage appears to differ in mice forced to run compared to those allowed to run. In our earlier study, forced running on a flat belt treadmill enhanced the knee joint osteoarthritic changes[37]. Running at a constant and predetermined speed, though not very high, may have caused a joint injury occasionally. In addition, there is some evidence that group caging, as in our earlier study, may predispose mice to joint accidents or traumas[42,43]. Group caged mice have less space for cage activities other than fighting. This might also affect the serum levels of stress hormones. In the present study, the mice lived in individual cages and had no chance of fighting. Decreased running activity in the older animals did not reflect the progression of OA or joint inflammation. In humans, this has been observed as well as in some experimentally induced models of arthritis in hamsters[44,45]. The running activity of normal mice was approximately 40% higher compared to the Col2a1 heterozygous knockouts. This did reflect to some degree the body weights of the mice. Physically smaller Col11a2 homozygous knockouts, weighing, on average, 10 g less than the normal mice or

the Col2a1 heterozygous knockouts, ran comparable distances despite their smaller size. Lifetime wheel running activity or changes in this activity towards the end of the experiment did not correlate with the OA scores. Wheel running may have substituted other forms of energy consuming behaviors, such as climbing and feeding on the lid, grooming and locomotion on the cage floor. Runner mice spent less time in these activities, which probably also explains the small differences in the weight development and feed intake[46].

C57Black/6J mice have often been employed in many investigations, as they have a high rate of spontaneous knee joint OA at old age, in up to 80% of mice at the age of 18 months[37]. In the present study, approximately 45% of normal mice had mild osteoarthritic changes in the tibial condyles and 20–40% in the femoral condyles at the age of 15 months. Running slightly increased the incidence of OA in tibial condyles but reduced it in the femoral condyles (differences not statistically significant). The heterozygous inactivation of Col2a1 gene in mice made the articular cartilage more susceptible to OA. The osteoarthritic changes in heterozygous knockouts occurred relatively early in life, by 9 months of age at the tibial condyles, compared with the normal mice. Later, the femoral condyles of the knockout mice were also affected. The osteoarthritic changes were in most cases limited to the uncalcified cartilage only. At the age of 15 months deeper lesions also could be observed. A positive correlation between the variation of body weight and the OA score was observed in the medial tibial condyles of the normal controls and runners as well as in the medial femoral condyles of normal runners. The connection between the body weight and knee joint OA in mice, is reminiscent of a similar correlation in humans[22,23,47].

Unexpectedly, lifelong physical exercise reduced the incidence of OA in the knockout mice. This effect could be seen as a trend also in the femoral condyles of the normal mice. Why wheel training decreased the prevalence of OA in gene-deficient mice is not known. Wheel running may have provided the mice with physiologically suitable locomotion for strengthening the muscles and ligaments around the knee joint, building up improved dynamic stability and shock absorbing capacity needed during joint loading and movements, but the situation was the same for control animals, too. Running training may have had an effect on the structure and strength of the collagen network of articular cartilage, especially in the knockout mice, where the organization of the intermediate and deep zone collagen network appeared to be inferior to that in control mice, judged from reduced birefringence. In osteoporosis, it has been suggested that the softer bones of the patients protect them from OA[48,49]. Interestingly, also in the Col2a1 knockout mice, the proximal humeral articular of cartilage was softer ($P=0.002$) and the volume fraction of the subchondral bone smaller ($P=0.01$) than in the controls (Hyttinen *et al.*, unpublished observations). This probably makes the knockout mice bone ends more compliant than in the wild-type animals. It is also possible that all these factors together, combined with lower body weight and shorter daily running distances, protect articular cartilage against injury better than other simultaneous physical activity in the cage. A reduction in the rate of OA after wheel running has been observed earlier in young hamsters[50] and inbred mice[51].

In conclusion, heterozygous knockout of Col2a1 gene increased the OA prevalence in C57BL/6J mice. Mice with homozygous knockout of Col11a2 gene did not have more OA than the C57BL/6J control mice. Lifelong voluntary wheel running reduced OA in both lines of knockout mice. The reason for this remains unknown. Reduction of OA may result from the strengthening of articular cartilage, i.e. reorganization and improved properties of collagen network or adjacent joint structures due to running. Furthermore, reduced body weight, shorter daily running distances and the increased compliance of the articular cartilage and bones of the knockout mice can contribute to the reduction of OA in exercised knockout mice.

Acknowledgments

This study was supported by the Research Foundation of Orion Corporation, Yrjö Jahnsson Foundation, North Savo Fund of the Finnish Cultural Foundation, and the Ministry of Education in Finland.

The authors are grateful for the skillful technical assistance of Ms Eeva Rahunen and Mr Kari Kotikumpu, as well as for the care of mice to the staff of the National Laboratory Animal Center, Kuopio, Finland.

References

1. Williams C, Jimenez S. Genetic and metabolic aspects. In: Reginster J-Y, Pelletier J-P, Martel-Pelletier J, Henrotin Y, eds. Osteoarthritis—Clinical and Experimental Aspects. Berlin: Springer 1999:134–56.
2. Holderbaum D, Haqqi TM, Moskowitz RW. Genetics and osteoarthritis: exposing the iceberg. Arthritis Rheum 1999;42:397–405.
3. Aszódi A, Pfeifer A, Wendel M, Hiripi L, Fässler R. Mouse models for extracellular matrix diseases. J Mol Med 1998;76:238–52.
4. Prockop DJ, Ala-Kokko L, McLain DA, Williams C. Can mutated genes cause common osteoarthritis? Br J Rheumatol 1997;36:827–30.
5. Kuivaniemi H, Tromp G, Prockop DJ. Mutations in fibrillar collagens (types I, II, II, and XI), fibril associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 1997;9:300–15.
6. Cicuttini FM, Spector TD. What is the evidence that osteoarthritis is genetically determined? Baillière's Clin Rheumatol 1997;11:657–69.
7. Vikkula M, Olsen B. Unravelling the molecular genetics of osteoarthrosis. Annals Med 1996;28:301–4.
8. Vandenberg P, Vuoristo M, Ala-Kokko L, Prockop DJ. The mouse col11a2 gene. Some transcripts from the adjacent rxr-β gene extend into the col11a2 gene. Matrix Biol 1996;15:359–67.
9. Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995;64:403–34.
10. Talts JF, Pfeifer A, Hofmann F, Hunziker EB, Zhou XH, Aszódi A, et al. Endochondral ossification is dependent on the mechanical properties of cartilage tissue and on intracellular signals in chodrocytes. Ann N Y Acad Sci 1998;857:74–85.
11. Savontaus M, Metsäranta M, Vuorio E. Retarded skeletal development in transgenic mice with a type II collagen mutation. Am J Pathol 1996;149:2169–82.

12. Li S-W, Prockop DJ, Helminen H, Fässler R, Lapveteläinen T, Király K, *et al*. Transgenic mice with targeted inactivation of the Col2a1 gene for collagen II develop a skeleton with membranous and periosteal bone but no endochondral bone. Genes Devel 1995;9:2821–30.
13. Helminen HJ, Kiraly K, Pelttari A, Tammi MI, Vandenberg P, Pereira R, *et al*. An inbred line of transgenic mice expressing an internally deleted gene for type II procollagen (Col2a1). Young mice have a variable phenotype of a chondrodysplasia and older mice have osteoarthritic changes in joints. J Clin Invest 1993;582–95.
14. Metsäranta M, Garofalo S, Decker G, Rintala M, deCrombrugghe B, Vuorio E. Chondrodysplasia in transgenic mice harboring a 15-amino acid deletion in the triple helical domain of pro-alpha-1(II) collagen chain. J Cell Biol 1992;118:203–12.
15. Hagg R, Hedbom E, Mollers U, Aszodi A, Fässler R, Bruckner P. Absence of the alpha-1(IX) chain leads to a functional knock-out of the entire collagen IX protein in mice. J Biol Chem 1997;272(33):20650–4.
16. Olsen BR. Collagen IX. Int J Biochem Cell Biol 1997;29:555–8.
17. Kimura T, Nakata K, Tsumaki M, Miyamoto S, Matsui YSE. Progressive degeneration of articular cartilage and intervertebral discs. An experimental study in transgenic mice bearing type IX collagen mutation. Int Orthop 1996;20:177–81.
18. Haimes HB, Jimenez PA, Li Y, Shinya T, Olsen BR. Overexpression of the NC 4 domain of type IX collagen induces ostearthritis in mice. Inflamm Res 1995;44(suppl. 2):S127–8.
19. Fässler R, Schnegelsberg PN, Dausman J, Shinya T, Muragaki Y, McCarthy MT, *et al*. Mice lacking alpha-1(IX) collagen develop noninflammatory degenerative joint disease. Proc Natl Acad Sci 1994;91:5070–4.
20. Nakata K, Ono K, Miyazaki J, Olsen BR, Muragaki Y, Adachi E, *et al*. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing alpha-1-(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993;90:2870–4.
21. Vandenberg P, Khillan JS, Ren Z-X, Mayne R, Li S-W, Arita M, *et al*. Transgenic mice with inactivation of the COL11A2 gene demonstrate a minimal cartilage phenotype at birth and at 7 months. Matrix Biol 1996;15:180.
22. Dieppe P. Osteoarthritis: time to shift the paradigm. Br Med J 1999;318:1299–300.
23. Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997;350:503–9.
24. Cooper C, Coggon D. Physical activity and knee osteoarthritis. Lancet 1999;353:2177–8.
25. McAlindon TE, Wilson PWF, Aliabadi P, Weissman B, Felson DE. Level of physical activity and the risk of radiographic and symptomatic knee osteoarthritis in the elderly: the Framingham study. Am J Med 1999;106:151–7.
26. Lane NE, Hochberg MC, Pressman A, Scott JC, Nevitt MC. Recreational physical activity and the risk of osteoarthritis of the hip in elderly women. J Rheumatol 1999;26:849–54.
27. Spector TD, Harris PA, Hart DJ, Cicuttini FM, Nandra D, Etherington J, *et al*. Risk of osteoarthritis associated with long-term weight-bearing sports: a radiologic survey of the hips and knees in female ex-athletes and population controls. Arthritis Rheum 1996;39:988–95.
28. Kujala UM, Kettunen J, Paananen H, Aalto T, Batie MC, Impivaara O, *et al*. Knee osteoarthritis in former runners, soccer players, weight lifters and shooters. Arthritis Rheum 1995;38:539–46.
29. Lindberg H, Roos H, Gardsell P. Prevalence of coxarthrosis in former soccer players. 286 players compared with matched controls. Acta Orthop Scand 1993;64:165–7.
30. Hannan MT, Felson DT, Anderson JJ, Naimark A. Habitual physical activity is not associated with knee osteoarthritis: the Framingham study. J Rheumatol 1993;20:704–9.
31. Lequesne MG, Dang N, Lane NE. Sport practice and osteoarthritis of the limbs. Osteoarthritis Cartilage 1997;5:75–86.
32. Kiviranta I, Tammi M, Arokoski J, Jurvelin J, Säämänen A-M, Parkkinen JJ, *et al*. Effects of mechanical loading and immobilization on the articular cartilage. Baillière's Clinical Orthopaedics 1997;2:109–22.
33. Helminen H, Kiviranta I, Säämänen A-M, Jurvelin JS, Arokoski J, Oettmeier R, *et al*. Effect of motion and load on articular cartilage in animal models. In: Kuettner K, Schleyerbach R, Peyron J, Hascall V, Eds. Articular cartilage and osteoarthritis. New York: Raven Press 1992:501–10.
34. Tammi M, Paukkonen K, Kiviranta I, Jurvelin J, Säämänen A-M, Helminen HJ. Joint loading induced alterations in articular cartilage. In: Helminen HJ, Kiviranta I, Säämänen A-M, Tammi M, Paukkonen K, Jurvelin J, eds. Joint Loading—Biology and Health of Articular Structures. Bristol: Wright 1987:64–88.
35. Kirkwood TB. What is the relationship between osteoarthritis and aging? Baillière's Clin Rheumatol 1997;11(4):683–94.
36. Hamerman D. Clinical implications of osteoarthritis and aging. Ann Rheum Dis 1995;54(2):82–5.
37. Lapveteläinen T, Nevalainen T, Parkkinen JJ, Arokoski J, Király K, Hyttinen M, *et al*. Lifelong moderate running training increases the incidende and severity of osteoarthritis in the knee joint of C57BL mice. Anat Rec 1995;242:159–65.
38. Lapveteläinen T, Tiihonen A, Koskela P, Nevalainen T, Lindblom J, Király K, *et al*. Training a large number of laboratory mice using running wheels and analyzing running behavior by use of a computer-assisted system. Lab Anim Sci 1997;47:172–9.
39. Busler DE, Li S-W. Rapid screening of transgenic type II and type XI collagen knock-out mice with three-primer PCR. Biotechniques 1996;21:1002–4.
40. Li Y, Lacerda DA, Warman ML, Beier DR, Yoshioka H, Ninomiya Y, *et al*. A fibrillar collagen gene, Col11a1, is essential for skeletal morphogenesis. Cell 1995;80:423–30.
41. Brunner H, van Beersum S, Warman M, Olsen B, Ropers H-H, Mariman E. A Stickler syndrome gene is linked to chromosome 6 near COL11A2 gene. Hum Molec Genet 1994;3:1561–64.
42. Weinreich S, Capkova J, Hoebe-Hewryk B, Boog C, Ivanyi P. Grouped caging predisposes male mice to ankylosing entesopathy. Ann Rheum Dis 1996;55:645–7.
43. Weinreich S, Hoebe-Hewryk B, Boog C, Ivanyi P. Solitary caging protects mice from ankylosing

entesopathy. Clin Rheumatol 1996;15(Suppl. 1): 32–3.
44. Walker DJ, Heslop PS, Kay LJ, Chandler C. Spontaneous ambulatory activity as a quantifiable outcome measure for osteoarthritis of the knee. Br J Rheumatol 1998;37:969–71.
45. Otterness IG, Bliven ML, Milici AJ, Poole R. Comparison of mobility changes with histological and biochemical changes during lipopolysaccharide-induced arthritis in the hamster. Am J Pathol 1994;144: 1098–108.
46. Harri M, Lindblom J, Malinen H, Hyttinen M, Lapveteläinen T, Eskola S, *et al*. Effect of access to a running wheel on behavior of C57BL/6J mice. Lab Anim Sci 1999;49:401–5.
47. Martel-Pelletier J. Pathophysiology of osteoarthritis. Osteoarthritis Cart 1998;6:374–6.
48. Dequeker J. The inverse relationship between osteoporosis and osteoarthritis. Adv Exp Med Biol 1999;55:419–22.
49. Dequeker J. Inverse relationship of interface between osteoporosis and osteoarthritis. J Rheumatol 1997;24:795–8.
50. Otterness IG, Eskra JD, Bliven ML, Shay AK, Pelletier J-P, Milici AJ. Exercise protects against articular cartilage degeneration in the hamster. Arthritis Rheum 1998;41:2068–76.
51. Lanier RR. The effects of exercise on the knee-joints of inbred mice. Anat Rec 1946;94:311–21.